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INTRODUCTION

A limited number of detailed studies on the development of the bovine testis from birth to sexual maturity have been reported (*12, 14*). Further study of bovine testis development appears desirable to furnish additional observations and to note variations that may occur during the developmental process. Information on the process of spermatogenesis should furnish a basis for experimental work designed to promote increased spermatogenic activity in the postpubertal testis as well as for work directed toward promoting complete spermatogenesis at an earlier than usual age.

This report is a study of the histological picture of the testes from 56 bulls varying from one to 83 weeks in age.

MATERIALS AND METHODS

Thirty-one Holstein, 8 Guernsey, and 17 Jersey bulls were studied. Unilateral castrations were performed on a particular bull at a specific age and the second testis removed two weeks later, except as noted. In general, no complications followed the operation except in one case where a severe screw worm infestation became established in the wound following the removal of the first testis. This case will be discussed in some detail.

Body weight of the bull was taken at the time of castration. Immediately following castration, the tunica vaginalis and other adnexa were removed and the spermatic cord was trimmed close to the testis proper. The epididymis was left intact. The testis was weighed and placed in a 10 percent formalin (4 percent formaldehyde) solution. Calcium carbonate was added to neutralize acidity. The smallest testes fixed well in toto (up to one to two inches in length), but the larger ones were gashed deeply in two or three places before fixing to allow penetration of the fixative to the deeper tissues.

Two samples of the smaller testes were taken (up to about two and one-half centimeters in diameter) for sectioning and observation. One sample was a transverse slice of the distal portion including the adjacent caudal epididymis and ductus deferens. The other sample was a proximal transverse slice and included a portion of the caput epididymis. Thus, a complete cross section of the testis from the outer tunica albuginea through the tubuli contorti, the tubuli recti, and the centrally located mediastinum, was available for study.

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Three samples of the larger testes were taken. Blocks approximately 2 by 3 centimeters by 1 centimeter thick were taken. One was from the distal area and included a portion of the adjacent caudal epididymis and ductus deferens. A second, from the proximal portion, included a part of the adjacent caput epididymis. The third block taken from each epididymis was a transverse section midway down the mediastinum and included a sample of this structure, a portion of the tubuli recti and a sample of the mesial tubuli contorti. These portions were washed and dehydrated by processing through increasing ethanol concentrations, cleared in xylene and embedded in paraffin (Fischer Tissue Mat), melting point 50 degrees C.

The tissues so prepared were sectioned at 5 and 10 microns. The deparaffined sections were stained with hematoxylin and eosin according to the method outlined by Guyer (*6*) and duplicates were stained with Mallory triple connective tissue stain as outlined in Bensley and Bensley (*2*). The hematoxylin-eosin stain was most useful in the study of cytological elements of the seminiferous tubules; the Mallory stain in the study of connective tissue elements, the interstitial space elements and blood cells. Stained sections were mounted in Permount and covered with a number one coverslip. Observation of these sections representing various portions of the testis from bulls varying in age from one week to 83 weeks was made using low, high dry, and oil immersion magnification. Measurement of the diameter of the seminiferous tubules was undertaken and was probably accurate in the early stages of testicular development but in the testes of the older bulls this measurement was not accurate because of the crowding effect among the tubules which distorted the round cross sections.

A detailed review of the literature (1, 3, 5, 7, 8, 9, 15, and others) that deals with a histological study of testis development in the bull as well as in other species was made to aid in interpreting observations with bull testis material.

OBSERVATIONS

For clarity of understanding, developmental changes in the histology of the testis is divided into four stages. The first stage is designated as the neonatal stage which extends from birth of the calf to about the 14th week of life. The neonatal stage is characterized by changes occurring in the testis from birth to the definite appearance of lumenization of the seminiferous tubules and appearance of the primary spermatocytes.

Stage two (15th to 28th week) is called the prepubertal stage. It is characterized by the presence of lumina in the tubules and by differentiation of the cytological elements of the seminiferous tubules to the first appearance of secondary spermatocytes and spermatids.

Stage three (29th to 36th week) is called the circumpubertal stage. During this phase of development there is completion of spermatogenic development with the presence of fully formed spermatozoa

within the lumen of the seminiferous tubules, within the lumina of the epididymis and within the ductus deferens.

Stage four (37th to 83rd week) is called the postpubertal stage. In this stage there is no further differentiation of the cytological picture but an increased and continued hyperplasia of the testicular elements with increasing age and body weight of the male.

The line of demarcation between each of these stages is arbitrary and ill-defined. There is considerable overlapping between bulls at similar ages and between testes of the same bull and even some within the same testis. Following is a detailed description of the histology of the bovine testis during the four stages.

The Neonatal Stage

At about one week after birth of the male calf, the seminiferous areas of the testis show a row of widely spaced nuclei that lie adjacent to the circumference of the occluded tubule. These nuclei are mostly granular. These appear to be the typical undifferentiated primordial cells of the seminiferous tubules and are the precursors of the spermatogonia and sustentacular cells, although there is no differentiation at this early age. There are other types of nuclei at this time. Amorphous nuclei located centrally in the mesenchymal substance of the tubule are present and granular nuclei located centrally and amorphous nuclei located peripherally appear but less frequently than the peripherally located granular nuclei that are most abundant (Figure 1).

From the literature on testis histology and with study of the material of the ages available, it is concluded that the peripherally or centrally located cells with amorphous nuclei are senescent primordial germ cells. The granular nucleated cells not on the periphery are developing spermatogonia and thus precursors of primary spermatocytes which have not yet appeared at this early age. In the early stage there are usually 6 well defined nuclei, mostly of the granular type, in a typical cross section five micra in thickness. The mean tubule diameter at this stage was found to be 46 microns. The remainder of the primordial tubule is packed with a mesenchymal-like material and no evidence of lumenization is found.

Little noticeable change in the histological picture is apparent in testis specimens taken from two-, three-, and five-week-old bull calves. A specimen taken from the testis of a six-week-old calf presented a picture in which the large centrally located cells with granular nuclei are present in greater numbers than any of the specimens observed. The progressive increase in these elements in subsequent specimens from older bulls during the neonatal stage indicated that they are vital developmental constituents of the seminiferous tubules and at this stage of development are immediate precursors of the primary spermatocytes.

Also in the specimen from the six-week-old bull there was the first recognizable appearance of spermatogonia distinguishable from sus-

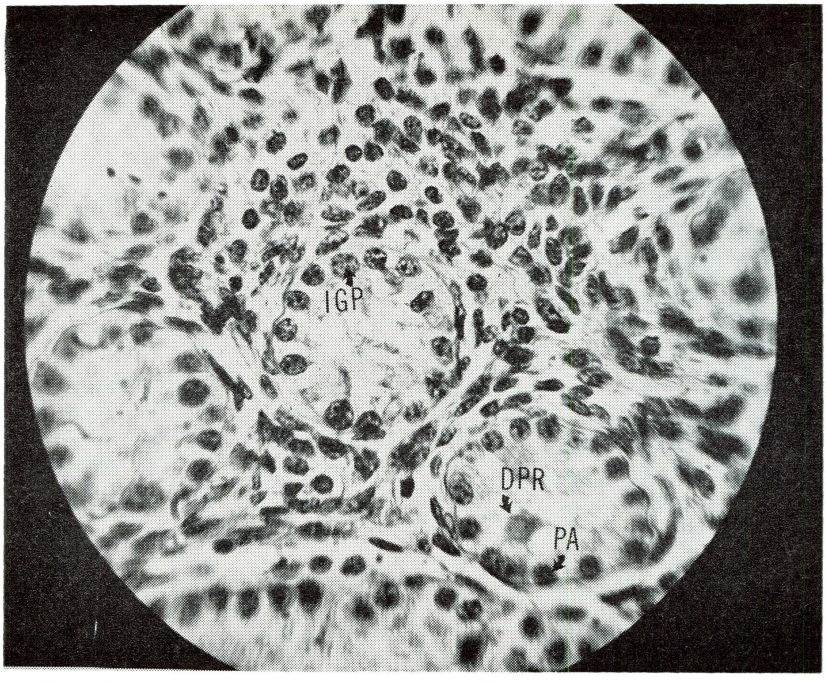


Figure 1. Early neonatal stage. X section 570X. Seminiferous tubules 1st week post partum. Note size of tubule cross section and abundance of interstitial material. IGP = Indifferent granulated-nuclear cells; DPR = Degenerating primordial cell; PA = Peripheral amorphous type cell.

tentacular cells. Both of these cell types have arisen from the non-differentiated primordial germ cells noted in the earlier aged specimens. Testicular specimens taken at later ages in the neonatal period showed some progressive development in this respect with beginning differentiation of primary spermatocytes (Figure 2).

In a specimen from a 14-week-old bull, there was definite differentiation of the primordial germ cells into spermatogonia and into primary spermatocytes. Also, there were true syncitial cells within the non-lumenized tubules. Thus, the development within the tubules at the end of the neonatal stage at about the 14th week of postnatal life in the bull consists of a single layer of circumferential cell lining the tubule with beginning development of primary spermatocyte differentiation and definite development of the sustentacular syncytium. There is no definite lumen within the tubule but even at 11 weeks there is a faint indication of the breakup of the dense centrally located mesenchymal material which presages lumenization.

During the neonatal stage, the proportion of interstitial space is large when compared to the space occupied by the tubules and it appears to be composed of undifferentiated mesenchymal cells primarily.

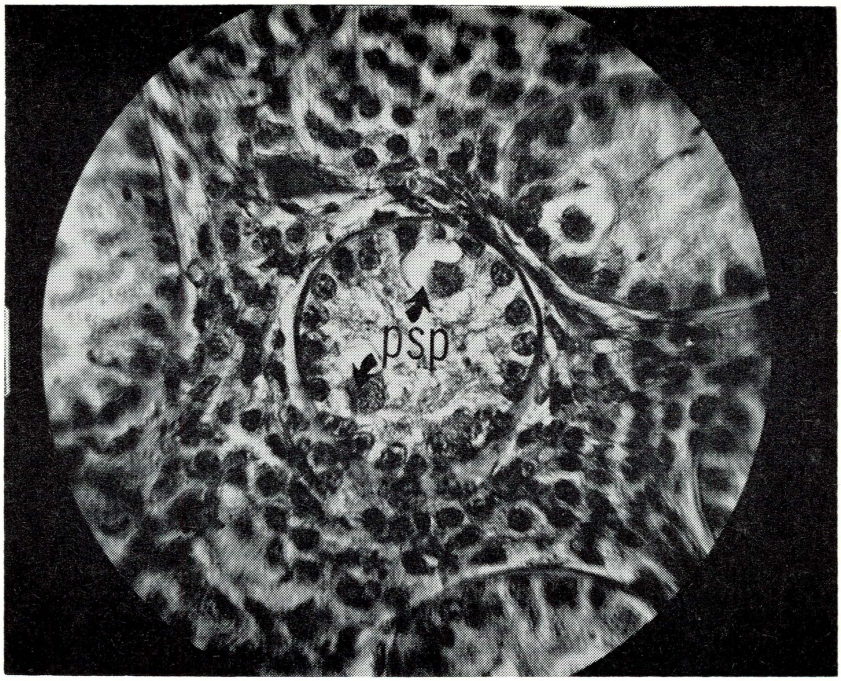


Figure 2. Mid-neonatal stage. X section 570X. Seminferous tubules 8th week post partum. Note crowding of cells and increased size of cross section. PSP = developing early primary spermatocyte.

However, even in a specimen from a one-week-old bull there is an indication that a few of the interstitial cells are functional Leydig cells. These appear similar to the cellular elements described by Gillman (*4*), Sniffen (*13*) and Nelson (*11*) in the testis of the human at birth. They believe these cells to be the result of stimulation of placental hormones during intrauterine life.

During the neonatal stage, the lacunae of the mediastinum and the lumina of the epididymal tubes and ductus deferens are patent. The ductus deferens is lined with a columnar epithelial lining and in at least one case this lining was differentiating toward a pseudostratified columnar type which is a characteristic of the structure in the mature genital tract.

During the neonatal period, the size of the testis increased from about 2 grams to approximately 25 grams in weight. There was an increase in the diameter of the seminiferous tubules from about 46 micra at the beginning of the period to about 75 micra at the end (14th week).

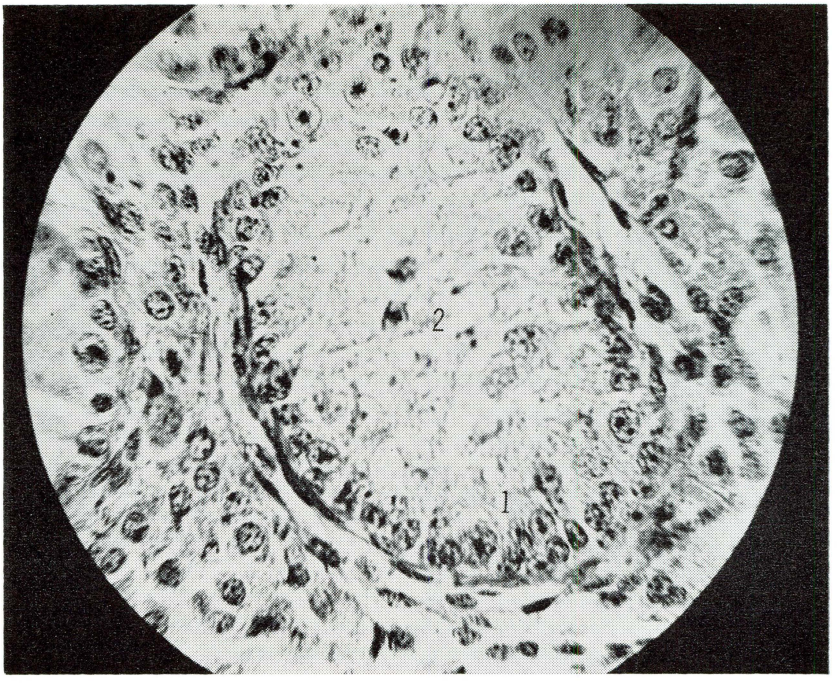


Figure 3. Early prepubertal stage—17th week. X section seminiferous tubule 570X. Note: 1—layering of cells; 2—breakup of central mesenchyme material.

The Prepubertal Stage (15th through 28th week)

Distinct lumenization of the seminiferous tubules was first observed in the testis from a 15-week-old Holstein. This specimen was also the earliest age in which mitotic figures were frequently present among the primary spermatocytes. In a specimen taken at the 17th week of life (Figure 3) the number of intratubular cells had become so numerous that more than one row of nuclei were disposed about the inner circumference of seminiferous tubules, yet there was not a definite two-row arrangement. The lumen was yet small and imperfectly formed. Many primary spermatocytes were present in various stages of division. A 19-week specimen showed further increase in cytological elements of the tubules and several layers of cells lined them. There was also increased size of the lumina of the tubules as well as increase in the diameter of the tubules (82 micra). Specimens of 19, 20, 21, 22, 23, 24, 25, 26, and 27 weeks showed that rapid hyperplasia was taking place during this period. Increased contortions of the seminiferous tubules, increased diameter of the seminiferous tubules (150 micra at 28 weeks), increased size of the lumen of the tubules with greater integrity of

the lumen, the presence of many mitotic figures, the presence of 4 to 5 layers of cells lining the tubules and the complete integrity of the sustentacular syncitium all are indicative of the rapid proliferation of the germinal epithelium during this period. A 28-week specimen observed showed the first secondary spermatocytes and some immature spermatids.

Variation in the developmental picture between the testes of the bulls of the same age, between tests of the same bull and within the same testis, increases as development proceeds.

In testes from bulls about 20 weeks of age there was a marked difference between the tubular development in the peripheral area (tubuli contorti) of the testes compared to that near the centrally located mediastinum (tubuli recti). The tubules were larger in diameter and there was a greater degree of compactness of the tubular tissue in the peripheral area than in the more centrally located areas. The looseness of the arrangement near the center of the testis can be noted at this age and later by the feel of the testis before fixation as well as by microscopic examination of these areas. Thus, there was variation with respect to the tubular development within the same testis even when the testis development may be considered normal.

Another source of variation within the same testis was the variation in adjacent areas due to the spermatogenic wave characteristic of spermatogenesis in the testis. Apparently, spermatogenesis proceeds longitudinally and cyclically along the seminiferous tubules and, whereas a specific section may show a complete cytological picture with respect to sperm production, an immediately adjacent section may show a less advanced and less active state of spermatogenesis. This is a normal variation and is present in sexually mature testes (*10*).

Several examples of variation between testes of different bulls of the same age were noted. Three specimens from 25-week Jersey bulls showed a definite lack of spermatogenic components in the seminiferous tubules as compared to similarly aged specimens. The germinal epithelium in these testes was composed primarily of sustentacular syncitium with sparse numbers of primary spermatocytes and few mitotic figures. The tubuli recti showed a greater degree of development than did the tubuli contorti which is the opposite to the situation that is observed in most instances.

The number of Leydig-like cells in the interstitium appeared to increase beginning at the 9th week and more or less coincided with increased libido at this time. There was in general a slight increase in the number of Leydig-like cells from the 19th week on. The proportion of interstitial space to tubular area decreased constantly as tubular proliferation progressed. With increasing size of the testis this decreasing proportion of intertubular space does not necessarily mean less total Leydig tissue. The size of the testis increased from about 25 or 30 grams to about 90 to 100 grams at the end of the prepubertal stage.

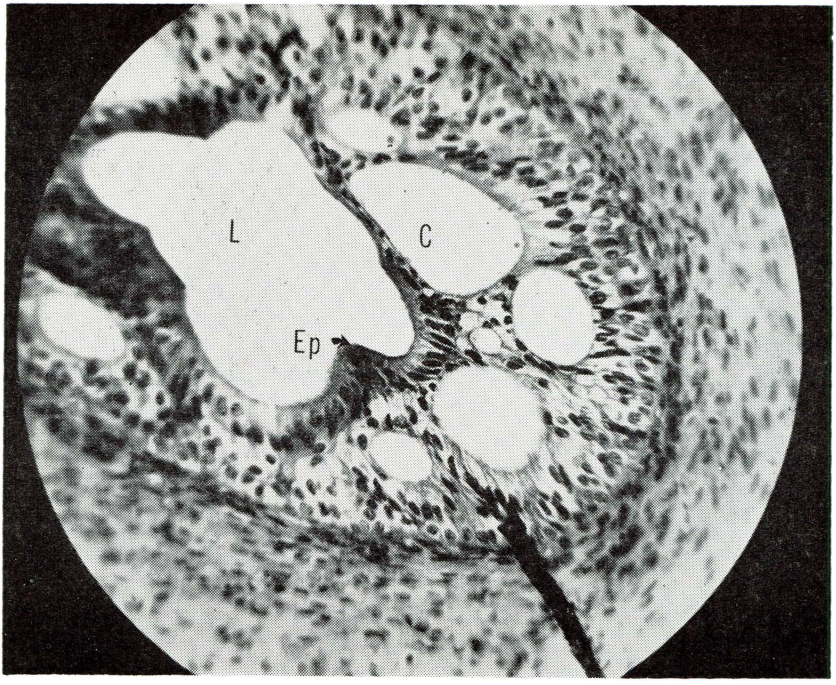


Figure 4. Cross section ductus deferens, 25th week post partum, 264X. L = lumen of duct; C = "canals"; Ep = typical epithelium of duct.

Cilia on the epithelial lining of the epididymal tubules were first observed at the 19th week. Canalization of the epithelial lining of the ductus deferens was noted in several specimens during this stage (Figure 4). Whether this is an abnormality of significance or not is unknown.

The Circumpubertal Stage (29th through 36th week)

This is a period of rapid progression in the spermatogenic activity of the testes in which there is differentiation of secondary spermatocytes into immature (tailless) spermatids, then into mature spermatids with tails and the production of free spermatozoa present in the lumina of the tubules, the epididymis and in the ductus deferens.

A 29-week specimen showed immature and tailless spermatids. However, some tubules near the mediastinum appeared to contain principally sustentacular syncitium with a sparse number of spermatocytes. Thus, different loci within the same testis again showed a contrast to the tubuli contorti where there was active cell division and spermatogenic components developed to the immature spermatid stage. In this peripheral area there were tightly packed tubules and

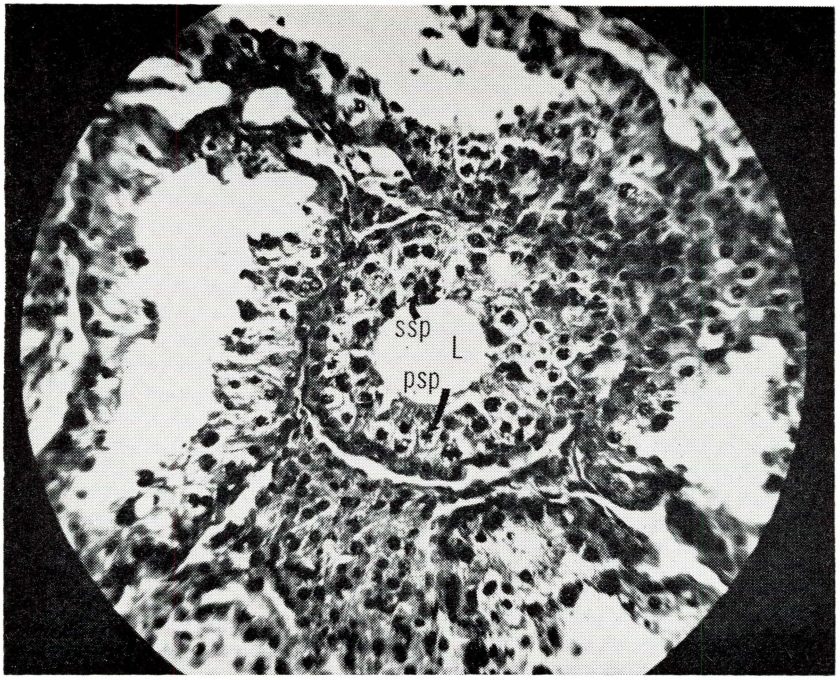


Figure 5. Mid-circumpubertal stage. X section seminiferous tubules 264X, 32nd week post partum. Note additional layers of cells and well developed lumen. L = lumen; PSP = primary spermatocyte in mitosis; Ssp = secondary spermatocytes.

decreased interstitial cell spaces. At 30 weeks a few tubules in some parts of the testis showed tailed spermatids attached to the sustentacular cells. Other sections of the same testis showed less progress in proximal loci, probably a result of the wave-like manner in which spermatogenesis takes place even after sexual maturity.

Figure 5 shows the condition in the circumpubertal period at 32 weeks. From this 32-week aged specimen to week 36, certain variations in spermatogenic development were noted. A specimen taken from a 36-week-old bull and which was considered to be normally developed (size and appearance) showed all stages of spermatogenesis, from spermatogonia, primary and secondary spermatocytes, spermatids, to free lying spermatozoa and masses of spermatozoa in the epididymal tubules and in the ductus deferens. Thus, full sexual development as far as qualitative change in the intratubular contents are concerned is attained at about the 36th week of life (Figure 6).

The epithelial lining of the epididymal tubules at 36 weeks is of the pseudostratified columnar type and is ciliated as in the sexually mature animal.

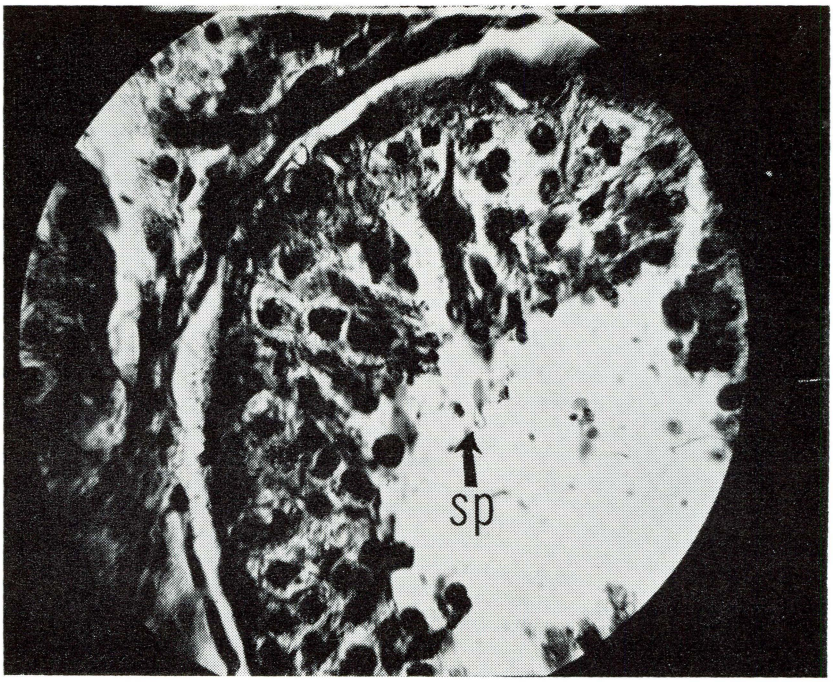


Figure 6. Late circumpubertal stage. X section seminiferous tubules 570X, 36 weeks post partum. All spermatogenic elements are present. Note additional layering and Sp—spermatozoa.

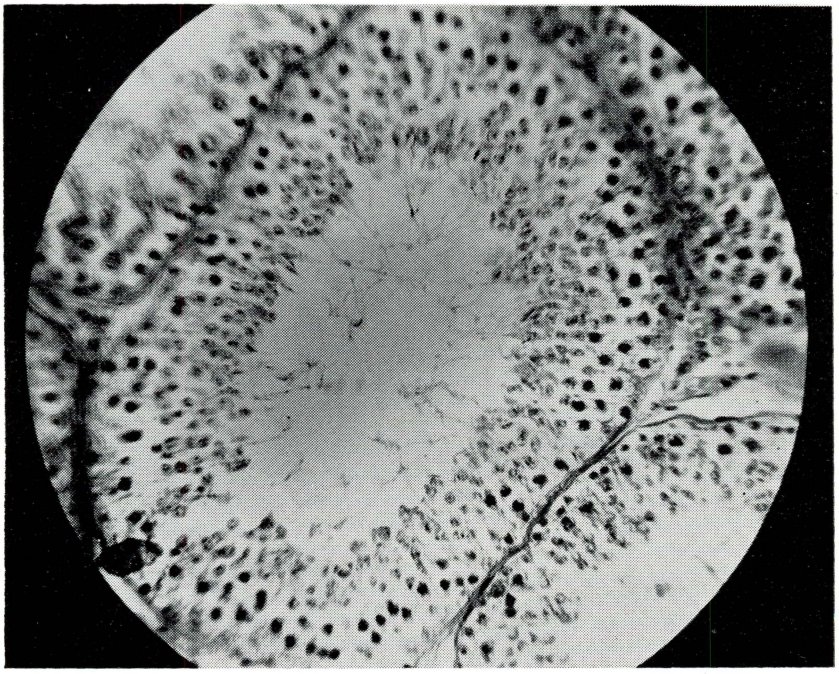
As noted above there are variations from the picture that we have considered to be normal (as judged by frequency of occurrence with respect to age and development of the bull).

A testis from a Holstein bull was removed at age 31 weeks. It was abnormally small—weight 35 grams, whereas the second testis removed two weeks later weighed 137 grams or somewhat above normal in weight for the age and size of the bull. The histological picture of the first removed 35-gram testis was similar to that of a normal specimen from a 23- or 24-week-old bull. Spermatogenesis had proceeded only as far as the primary spermatocyte stage. On the other hand the 137-gram testis from this bull removed at 33 weeks was much more advanced in intratubular development. In fact it was the earliest aged specimen observed that had free spermatozoa in the lumina of the seminiferous tubules. Thus, in this testis that was above normal in size for the age of the bull, there was apparently above normal intratubular spermatogenic development. Probably both testes were under similar hormonal influence from the pituitary yet one had failed to respond to the degree that the other one had with respect to hyperplasia and hypertrophy of testicular tissue.

The Postpubertal Stage (37th week to latest age observed—83 weeks)

From the 37th week onward, with the exception of certain specimens that varied from the usual development, there were no new developmental elements. Changes during this period were quantitative and impressive. From a mean testis weight of about 100 to 150 grams at 37 weeks there was an increase to about 300 grams at 83 weeks. Although the measurement of tubule diameter is inaccurate after the 26th week there appears to be some increase in diameter of the tubules during this stage. A mean diameter of about 200 micra was observed at the beginning of the period at 37 weeks whereas it was 250 micra in the later part of the period studied. We can only presume that testicular weight probably continued to increase to some extent until mature body size was attained. Mature tubule diameter has probably been attained at 80 weeks. Specimens from testes of the 69th week (Figure 7) show a complete cytological picture, spermatogonia, primary and secondary spermatocytes, spermatids and free spermatozoa. At this age the testis appears to be tightly packed with tubular components and the proportion of intersititial cell space is small due to the hyperplasia and hypertrophy of the tubular elements.

Figure 7. Full postpubertal development. Oblique section seminiferous tubule, 264X, 69th week post partum. Note enormous increase in diameter of seminiferous tubule.



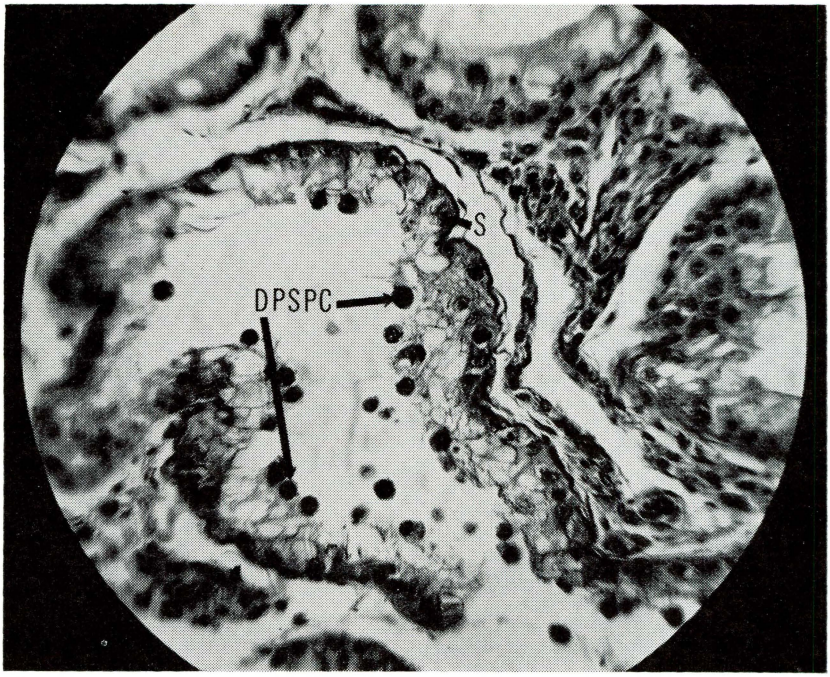


Figure 8. Postpubertal stage. X section seminiferous tubules following screw worm infestation between 50th and 52nd weeks. S = sertoli cells and syncytium; DPspc = degenerating primary spermatocytes. Note devastation to germinal epithelium.

Usually there are several cell layers within the seminiferous tubules but this varies some with the individual specimen. One specimen from a 42-week Guernsey bull showed less profuse development than the normal specimen of 38 weeks. Similarly, a specimen at 45 weeks from a Holstein showed a histological picture in which only 2 or 3 layers of cells were present in the tubules. There were large lumina and sparse occurrence of spermatids in this 45-week specimen. Both these bulls were undersized for their ages.

In the case of the bull from which the 45-week specimen was obtained, this undersized bull was subjected to better care and feeding for a period of 10 weeks after which time the other testis (specimen 55-week) was removed. This later removed testis showed considerable improvement in spermatogenic development compared to the specimen removed at 45 weeks. Whether the improvement was due to the improved feeding regime or to inherent variability between the two testes is impossible to conclude.

Another specimen that varied drastically from the normal during this stage was that from a 52-week-old Jersey. At 50 weeks the first

testis was removed and it was apparently normal. Following castration a severe screw worm infestation of the wound occurred. The entire animal was affected and the second testis removed at 52 weeks showed that complete degeneration of the germinal epithelium and other cytological elements concerned with spermatogenesis had taken place. Nothing remained except the sustentacular syncytium (Figure 8). Thus, development prior to the infection had completely degenerated in the short time between the instigation of infection and removal of the second testis. The weight of the second testis was 108 grams compared to 123 grams for the first one removed.

SUMMARY

Histological changes in the testis of the bovine from birth to 83 weeks of age were studied.

Definite lumenization of the tubules and the appearance of definite primary spermatocytes occurred at about the 14th postnatal week.

Secondary spermatocytes and spermatids appeared at about 28 weeks postnatally. Fully formed spermatozoa were first observed in testicular specimens from bulls between the ages of 29 and 36 weeks.

Considerable variation in the progression of testis development was apparent between bulls of similar age, especially if the size of the animal or the testis was abnormal.

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